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BOTANICAL GAZETTE

AUGUST, 1903

STUDIES IN SPINDLE FORMATION.

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(WITH PLATES XV AND XVI)

HISTORICAL.

THE evidence produced from the researches of the last few years¹ proves quite conclusively that a centrosome as a spindle-forming organ does not exist in the higher plants. Too few forms, however, have been worked out in sufficient detail to allow of any definite conclusions as to whether or not these plants have any common uniform method of spindle formation. Of the types that have been thoroughly examined, the following have been recorded:

In 1897 Osterhout investigated the spindle in the spore mother-cells of *Equisetum*. The first indication of a spindle in these cells is the formation of a felted zone of kinoplasmic fibers surrounding the nucleus. These fibers grow out from the nuclear membrane and assume a radial position. By the fusion of their free ends these fibers form a series of cones, and upon the breaking down of the nuclear membrane the cones unite at their apices in two groups to form the bipolar spindle.

In 1898 the writer investigated the development of the spindle in the pollen mother-cells of *Cobaea*. Here it was found that, as division approaches, a dense granular cytoplasmic substance

¹ Belajeff (1894), Byxbee (1900), Davis (1899, 1901), Debski (1897), Farmer (1893, 1895), Guignard (1898), Juel (1897), Lawson (1898, 1900), Mottier (1897 *a, b*), Némec (1898, 1899), Osterhout (1897, 1902), Smith (1900), Strasburger (1896, 1897, 1900), Webber (1897), Weigand (1899), Williams (1899).

forms a complete zone about the nucleus. The nuclear wall disappears, and the central part of the cell becomes filled with a network of kinoplasmic fibrils, in which the chromosomes lie. This network grows out into several projections, which become the primary cones of the multipolar spindle. These cones now unite at their apices into two groups and thus form the bipolar spindle.

In 1899 Williams describes for *Passiflora* a process somewhat resembling that which occurs in *Cobaea*. Upon the breaking down of the nuclear wall, there is a large kinoplasmic network formed in the central portion of the cell. This network of fibrils projects outward at various points and becomes cones, which, uniting at their apices in two groups, form the mature spindle.

In 1900 the writer investigated the spindle in *Gladiolus*. Here, as in *Equisetum*, the first indication of the spindle in the mother-cell is the formation of a felted zone of kinoplasmic fibrils surrounding the nucleus. But instead of the fibrils taking on a radial arrangement, as described for *Equisetum*, the zone grows out at irregular intervals in the form of sharp pointed projections, which are the primary cones of the spindle. The nuclear wall remains intact until the cones are fully developed, and upon its breaking down the cones collect by the fusion of their apices into two groups, when the bipolar condition is reached.

Byxbee (1900) has recorded the following method for the development of the spindle in the pollen mother-cells of *Lavatera*. The meshes of the network close to the nuclear wall pull out in a direction parallel to the wall, forming a felt of fibrils about the nucleus. The cytoplasm now collects in a dense granular zone about the nuclear membrane, as it does in *Cobaea*. Upon the breaking down of the nuclear wall, there is formed a central mass of fibrils in which the chromosomes are suspended. This central mass of fibrils grows out into several projections, bringing about the multipolar condition of the spindle. Two of the cones become more prominent than the others, which they absorb, and the bipolar figure is thus produced.

Smith (1900) has worked out with considerable detail the

development of the spindle in the spore mother-cells of *Osmunda*. In this case the spindle originates from a zone of kinoplasm which surrounds the nucleus. The granules in the kinoplasm arrange themselves in rows, concentric with the nuclear wall, and are finally massed on opposite sides of the nucleus. From these masses two cones of fibrils are developed, which become the two cones of the spindle. As there are only two primary cones developed, the spindle is bipolar from the first.

Osterhout (1902), in his recent work on *Agave*, records the following series of events leading to the formation of the spindle in the mother-cell. During the early stages there is developed a cytoplasmic membrane outside of the nuclear wall. This he calls the "spindle membrane," and he regarded it as a unique structure. There is no weft of fibrils formed, but the spindle-forming fibrils are radial from the beginning, and are attached to both the nuclear and spindle membranes. From these radial fibrils the cones are developed, and these, by fusing into two groups, bring about the bipolar condition. Probably the most remarkable observation that Osterhout has recorded is that the second spindle formation differs entirely from the first. According to his observations, the spindle-forming fibrils of the second division are found in close contact with the nuclear wall, and their free ends radiate from it. They extend outward into the cytoplasm, and by the union of their ends form a series of cones. These fuse at their apices into two groups and form the bipolar spindle in much the same fashion as in *Equisetum*.

From the observations of the writers described above it is perfectly obvious that there are considerable differences between the methods of spindle formation. It is also evident that there are certain important features which are common to nearly all of them. It would seem that there are several distinct types of spindle formation, but the differences between them are too great, and the number of forms worked out in detail are too few to allow of any generalizations. The number and character of these types can be determined only by additional observations. It is to this end that the following studies are recorded.

METHODS.

These were essentially the same as those used in my work on *Cobaea* and *Gladiolus*. As full details are recorded in these papers, it will be unnecessary to repeat them. It is only necessary to add that the material was all fixed in the field, and by far the most satisfactory results were obtained by using Fleming's weak solution of chromic-osmic-acetic acid as a killing agent, and the triple stain, safranin, gentian, and orange G.

THE POLLEN MOTHER-CELLS OF *IRIS FLORENTINA*.

The young anthers of the common garden *Iris* furnish very good material for the study of spindle formation. If conditions are favorable and the material is fixed in the field, immediately after being detached from the plant, all the stages in the formation of the spindle of the first division of the pollen mother-cells may be observed in a single anther. As the anthers at this stage are very large, many sections may be obtained from one of them. Previous to the formation of the first spindle, the nucleus of the mother-cell is very large, containing one or two nucleoli and the chromatin thread. The cytoplasm appears to be a reticulum, the threads of which are more or less granular. In the immediate neighborhood of the nuclear wall, the cytoplasm is more dense than at the periphery, but this dense region is not as sharply differentiated as that described by the writer for this stage in the pollen mother-cells of *Cobaea* and *Gladiolus*. Numerous small spherical bodies, probably oil globules, were found distributed irregularly through the cytoplasm.

While the chromatin is yet in the spirem stage, the cytoplasm immediately in contact with nuclear membrane becomes differentiated into a distinct weft or felted zone of fibrils. This weft appears at first to consist of a few delicate but very distinct fibrils, which stain blue with the gentian violet, in contrast to the slightly orange color of the rest of the cytoplasm. They do not radiate from the nuclear membrane, but lie more or less parallel to it. When followed along their course, they were found to lose gradually their affinity for gentian violet, and to terminate in the regular orange-colored threads of the cytoplasm. This indicates

that the kinoplasmic threads are nothing more than modified threads of cytoplasm, which have lost their reticulated and granular character and have become more distinctly thread-like. *Fig. 1* shows one of the early stages in the formation of the weft, which ultimately develops into the spindle. Even in stages earlier than this, the fibrils were sharply differentiated, both in their structure and staining properties, from the surrounding cytoplasm. The fibrils gradually increase in numbers, evidently at the expense of the reticulum of the cytoplasm, and soon a fibrous zone of considerable thickness completely surrounds the nuclear membrane *fig. 2*. As far as the development of this zone is concerned, the process is identical with that which occurs in *Gladiolus* (Lawson, 1900). In *Gladiolus*, however, the chromosomes were already formed when the weft was developing, while in *Iris* the chromatin is yet in the spirem stage.

Fig. 2 shows the appearance of the fibrous zone when fully developed. Up to this time it increased in thickness almost uniformly, but it now grows outward at irregular intervals. Two of these outward projections are shown in *fig. 2*. The meshes formed by the interlacing fibrils of the weft become elongated in the directions in which the fibrils are growing, that is, in the direction of the projection. These outgrowths of the fibrous zone are the first indications of the primary cones of the so-called multipolar stage of the spindle, and the threads composing them converge toward their apices.

During the development of the cones the chromatin assumes the form of distinct chromosomes, and the nucleoli become vacuolated.

The development of the cones is not only brought about by the pushing out of the original weft at certain intervals, but they apparently increase at the expense of the cytoplasm into which they project. *Fig. 3* shows one of the cones projecting into the cytoplasm, and the outermost fibrils converging to its apex are continuous with the fibrils of the cytoplasmic reticulum. *Fig. 4* shows the cones at a later stage; the larger cone is much more sharply defined. It has lost much of its reticulated appearance, and its fibrils are more independent of one another, except

where they converge at the apex. There is apparently no definite position for the projection of each cone. They may be separated by considerable distance, or again there may be two or three quite close together (*figs. 5, 6*). The number also seems to vary, although there are always more than two formed. Cross sections always show three or four. *Fig. 6* shows three on one side, and an indication of a fourth on the opposite side of the nucleus. This figure also shows that the growth of the various cones is not simultaneous.

One of the striking features of the cones is the remarkably sharp point with which each one terminates. These sharp-pointed apices extend outward as the fibrils composing the cones elongate, and they indicate the outermost points at which are taking place the changes which bring about the transformation of the cytoplasmic reticulum into spindle-forming fibrils.

As in *Gladiolus*, the nuclear membrane persists until the cones have almost attained their maximum development. In *fig. 7* a portion of the nuclear wall remains, and several of the cones have fused together before the nuclear membrane entirely disappears. This fusion, however, probably does not begin until the nuclear wall begins to break down. When this is accomplished the chromosomes become attached to the fibrils at the base of the cones. During and after this stage the collecting together of the cones was very noticeable.

By the time the nuclear wall has entirely disappeared, and all the chromosomes are connected with the fibrils, the cones unite at their apices into several groups (*fig. 8*). Here three of these groups are represented, which are the product of the union of several primary cones. The space that was occupied by the nuclear sap is now filled with a complex of delicate fibrils, and all of the chromosomes are connected with fibrils which extend to the apex of one or other of the cones. By further union of the cones (*figs. 9, 10*), the number of cone aggregates is reduced to two. Up to and including the stage represented in *fig. 9*, the fibrils composing the cones were of the same general character, but in the following stages there is a differentiation of the fibrils, according to the part they take in the mature spindle. We have,

for instance, those that are connected with the chromosomes, those that extend from end to end of the spindle, and those that extend laterally outward with their free ends projecting into the cytoplasm. These are known as the connective, continuous, and mantle fibers respectively.

The spindle having now reached the bipolar condition, and the connective fibrils from the respective poles having become connected with the chromosomes (*figs. 10, 11*), the latter bodies take up their characteristic position at the equator, and the formation of the spindle is complete.

The series of events leading to the formation of the spindle, as here described for *Iris*, agrees in every essential detail with those which occur in *Gladiolus*.

THE POLLEN MOTHER-CELLS OF *DISPORUM HOOKERI*.

The anthers and pollen mother-cells of *Disporum* are not as large as many other liliaceous types that have been used for the study of spindle formation ; nevertheless, they form an extremely interesting subject. When properly fixed in the field, the various stages in the formation of the first spindle of the mother-cell may be readily obtained.

In the resting condition of the mother-cell, the nucleus is centrally situated, but as division approaches it is invariably found near one side. Before any kinoplasmic differentiation takes place, the chromatin has broken up and assumed the form of definite spherical chromosomes, of which there are but eight. The first evidence of spindle formation is to be found in the transformation of the cytoplasm in the immediate vicinity of the nuclear membrane. At first, this change takes place in much the same manner as described above for *Iris*. There are in the beginning but a few short threads, which when followed outward are found to be continuous with the reticulum of the surrounding cytoplasm, but they have lost the granular character and stain blue with the gentian violet. These threads increase in number and gradually form a distinct weft, which appears to form more abundantly on one side of the nucleus than on the other ; that is, it is much more evident on the side

of the nucleus farthest away from the cell wall (*fig. 12*). While the beginning of the weft resembles that described for the stage in *Iris*, it soon takes on a very different form as it increases in size. Instead of the meshes running more or less parallel to the membrane of the nucleus, they elongate more at right angles to it (*figs. 12, 13*). The fibrils composing the weft stain deep blue, while the peripheral cytoplasm stains slightly orange. If, however, we follow the individual fibrils outward, they gradually lose their property of staining blue and stain slightly orange. They are, in fact, strictly continuous with the more granular threads of the cytoplasmic reticulum. They therefore seem to be nothing more than transformed threads of cytoplasm.

As already stated, the growth of the weft is not uniform; it is much more conspicuous on one side of the nucleus than on the other. This irregularity is carried still farther as the weft increases in size. The meshes elongate much more, the individual fibrils which form them lengthen considerably, and their individuality becomes much more pronounced. As the weft now increases, it does so by several projections, which terminate in sharp points, so that we have distinct cones formed (*fig. 14*), in much the same manner as they are formed in *Iris*. These cones at first vary considerably in number and size, and are invariably much more numerous on the side of the nucleus away from the cell wall (*fig. 14*). From a careful study of the development of these cones as they project into the cytoplasm, there seems to be little doubt that the fibrils composing them are transformed directly out of the reticulum of the cytoplasm. This is particularly evident in the earlier stages, where there is no sharp differentiation between the ends of the fibrils of the weft and the surrounding threads of cytoplasm, the one passing into and apparently being continuous with the other.

As the cones project outward, they terminate in very sharp points and extend over half way to the cell wall opposite (*figs. 14, 15*). During the entire process of their development the nuclear membrane and the nucleolus persist. There is no evidence of the breaking down of these structures until the cones are fully developed. As soon as the cones cease growing out-

ward, the nuclear membrane disappears rapidly, and the ends of the fibrils at the base of the cones become attached to the chromosomes.

The sequence of events which leads to the bipolar condition of the spindle is essentially the same as that described for *Iris*. Upon the breaking down of the nuclear membrane the apices of certain of the cones move toward each other and form several groups of cones. These in turn unite still further, until we have the characteristic so-called multipolar spindle. *Fig. 16* shows a stage approaching the bipolar condition. The connective fibers which attach themselves to the chromosomes become more sharply defined than the other fibrils, and appear to be thicker in the region of the chromosomes, as if they had already begun to contract. By the time the chromosomes have arranged themselves at the equatorial plate, the cones have united into two groups, which are the poles of the bipolar spindle. The mature spindle does not show the free mantle fibers which are so characteristic of *Cobaea*, *Gladiolus*, *Iris*, and many other forms. There are a few delicate continuous fibrils, which extend from pole to pole. The connective fibrils are much coarser than the continuous ones, stain more deeply, and are much more clearly defined. In the bipolar stage of the spindle, these fibrils appear to be much thicker in the region of the chromosomes, which suggests that they have not begun to contract, but that the chromosomes imparted the stimulus to contract. This condition of the fibrils is shown in *fig. 17*.

As the chromosomes (eight in polar view) approach the poles, the connective fibrils shorten and thicken, and by the time they have reached the poles the connective fibrils have disappeared entirely; the continuous fibrils, however, have increased in numbers. Having reached the poles, the chromosomes unite and form a mass of chromatin at each end of the spindle. Nuclear sap is now secreted, and a membrane is formed about each daughter nucleus.

The daughter nuclei are small, and the various stages in the development of the spindle of the second division were difficult to follow. The early weft stage, however, was observed, as well

as the later multipolar stages, which show clearly that the process of spindle formation in the second division is essentially the same as in the first.

THE POLLEN MOTHER-CELLS OF HESPERALOE DAVYI.

As in *Iris* and *Disporum*, the first indication of spindle formation in the pollen mother-cells in *Hesperaloe* is the differentiation of the cytoplasm in the immediate neighborhood of the nuclear wall into a distinct weft of fibrils. This weft is very small at first, but as it stains blue very readily when the triple stain is used, it can be clearly distinguished. The fibrils run almost parallel with the nuclear membrane, even at the beginning of their formation, and they keep this position until the weft has reached a considerable thickness. In this respect it is almost identical with *Gladiolus* and *Iris*, but differs slightly from *Disporum*. *Fig. 18* shows the weft fairly well developed. It increases uniformly and completely surrounds the nuclear membrane. As in *Iris* and in *Disporum*, there is no sharp differentiation between the outer fibrils and the reticulum of the surrounding cytoplasm. It would seem that the cytoplasm loses its granular structure, becoming more distinctly threadlike, with meshes parallel to the nuclear wall, and stains blue instead of orange.

Following the same series of events that occur in *Gladiolus*, *Iris*, and *Disporum*, the weft soon ceases its uniform growth and proceeds to grow out at irregular intervals in the form of pointed projections. The meshes of the weft in these outward growths are no longer parallel to the nuclear membrane, but are elongated in the direction of the projections. *Fig. 19* shows one of these projections. It also shows that the fibrils of the developing cone pass into the threads of the surrounding cytoplasmic reticulum, suggesting that they grow at the expense of the cytoplasm. As the cone pushes outward, the meshes elongate proportionately, and the fibrils composing them become much more sharply defined. As in *Iris* and *Disporum*, there appears to be no definite number of cones formed; there are usually four or five to be seen in section.

As the apices of the cones approach near to the cell wall, the nuclear membrane breaks down, and the space once occupied by the nuclear sap becomes filled with the ingrowing fibers of the base of the cones (*fig. 20*). By the time the nuclear wall has disappeared, some of the cones unite at their apices. This union continues in the same fashion as that described in *Gladiolus*, *Iris*, and *Disporum*. *Fig. 21* shows a condition in which the nuclear wall has entirely disappeared, and five cones are seen in section. Some of these cones are evidently the result of the union of several primary cones. By the time this stage is reached the fibrils have become long independent threads, converging to the apices of the cones, and many of them have become attached to the chromosomes.

The fusion of the cones is probably a very rapid process, as the multipolar stages were only obtained from material fixed in the field, immediately after being dissected from the plant. They are never found as frequently as the bipolar stage.

Fig. 22 shows the cones uniting into two groups, pointing in opposite directions. Before fusing, the cones point outward in all directions, so that in a section few of them show (*fig. 21*), but as they collect in groups a median section shows many more in the same plane. When the bipolar condition is finally reached, the usual three sets of fibrils are sharply differentiated. The connective fibrils are clearly defined and appear to be thicker in the region of the chromosomes. The continuous fibrils extend uninterruptedly from pole to pole and are much finer than the connective fibrils. Extending laterally from the poles, numerous mantle fibrils are to be seen, with their free ends projecting into the cytoplasm (*fig. 23*).

As shown by the series of figures, the process of spindle development in the first division of *Hesperaloe* is essentially the same as that described for *Gladiolus*, *Iris*, and *Disporum*. On account of the scarcity of the material, the development of the spindle for the second divisions was not observed.

THE POLLEN MOTHER-CELLS OF *HEDERA HELIX*.

From the above description, it seems quite evident that there is a very striking resemblance in the method of spindle forma-

tion in *Gladiolus*, *Iris*, *Disporum*, and *Hesperaloe*. As these types are representative of related families, it is not surprising to find such a resemblance. This method of spindle development, however, is not peculiar to these families, as the following descriptions of the conditions existing in *Hedera* will show.

The young anthers containing the pollen mother-cells of *Hedera helix* are extremely small and difficult to handle. But after being fixed in the field and imbedded, the difficulties are mostly overcome, for the mother-cells stain very easily with the triple stain, and the various stages in the development of the spindle of the first and second division are readily obtained.

The mother-cells in *Hedera* are much smaller than the liliaceous plants, but the nuclei are relatively much larger. As division approaches, the amount of nuclear sap is very great, and as a result the nucleus occupies one-half the space of the cell. Before any kinoplasmic differentiation takes place, the cytoplasm presents a uniform granular reticulum, but this appears to be slightly denser in the vicinity of the nuclear membrane, suggesting the condition that exists in *Cobaea* and *Gladiolus*. As soon as the chromatin has segmented to form the chromosomes, the cytoplasm in contact with the nuclear membrane becomes differentiated into a thin web of fibrils, which stain an intense blue. The development of the web is essentially the same as that described for *Gladiolus*, *Iris*, *Disporum*, and *Hesperaloe*. It consists at first of only a few threads, which interlace with each other and run more or less parallel to the nuclear membrane. The fibrils nearer the membrane stain a very deep blue, but those farther out stain less, and as they merge into the surrounding orange-staining cytoplasm, they are slightly granular and are no longer to be distinguished from the reticulum of the latter (*fig. 24*). The origin of the fibrils of the web is apparently due to the change in the structure of the threads of cytoplasm, as described in *Iris*, *Disporum*, and *Hesperaloe*. As in these plants, the web is uniformly thick in the early stages of its formation, but it soon develops projections at irregular intervals, producing the primary cones of the multipolar figure (*figs. 25, 26*). As the primary cones grow outward, the fibrils composing

them no longer run parallel to the nuclear membrane or interlace with one another. They become long independent fibrils, projecting more at right angles to the nuclear membrane and converging at the apices of the cones. *Figs. 26 and 27* show several of these cones, nearly fully developed.

As the cones approach the completion of their development, the nuclear membrane suddenly disappears, and the fibrils at the base of the cones come in contact with the chromosomes. The fusion of the cones proceeds until there are two groups (*fig. 28*). The chromosomes are very numerous, and the mature spindle is consequently very wide at the equator. The usual connective and continuous fibrils are to be distinguished, but the mantle fibrils do not appear until the chromosomes begin their migration to the poles.

It is quite clear from the series of stages shown in *figs. 24 to 29* that the formation of the first spindle in *Hedera* is similar to that which occurs in *Gladiolus*, *Iris*, *Disporum*, and *Hesperaloe* in every essential particular.

In his work on *Agave*, Osterhout describes two distinct types of spindle formation in the two divisions preceding the development of the pollen. In the second division the spindle originates in a way that is absolutely different from that in the first. There is no weft surrounding the nucleus in the early stages, but instead there is a series of fibrils which radiate out from the nucleus, with their free ends projecting into the cytoplasm. Such a stage as this does not occur in the first division, and it is remarkable that two conditions so essentially different could be found in two succeeding generations of cells. With the idea of ascertaining whether any such difference as this existed in the two succeeding divisions of the mother-cell in *Hedera*, a very careful examination was made of every stage in formation of the second spindle. This proved quite conclusively that the spindles of the first and second divisions are formed in identically the same fashion.

Very little time elapses between the first and second divisions. As soon as the first spindle reaches the bipolar stage, the chromosomes move to the respective poles and unite, forming

two masses of chromatin at opposite sides of the cell. While this is taking place (*fig. 30*) numerous long mantle fibrils extend from the sides of the masses of chromatin and at the same time the continuous fibrils increase in number. The mantle fibrils, however, are not confined to the lateral position on the chromatin mass, but radiate from all sides of it. These radiating fibrils persist for a considerable time, even after the chromatin has secreted a nuclear sap and surrounded itself with a membrane. *Fig. 31* shows two mature daughter nuclei, with the chromatin in the spirem stage, each surrounded by a distinct membrane. The continuous fibrils between the two nuclei begin to disappear in the equatorial region of the cell, and each nucleus is completely surrounded by a system of radiating fibrils with their free ends projecting into the surrounding cytoplasm. When first observed, the writer mistook this condition for the radiating stage that Osterhout has figured in the formation of the second spindle in *Agave*. The two conditions are strikingly alike, but a careful study of the stages immediately preceding and following this showed conclusively that the radiating fibrils were the remnants of the first spindle and not the beginning of the second. As shown in the next stage (*fig. 32*), these radiating fibrils and the continuous fibrils disappear completely, and take no part whatever in the formation of the second spindle. As *fig. 32* illustrates, the resting period of the daughter nuclei is a very short one. The chromatin breaks up into chromosomes before the last of the continuous fibrils have vanished.

The first evidence of the beginning of the new spindle is the transformation of the cytoplasmic reticulum close to the daughter nuclei into a web of fibrils completely surrounding each nucleus (*fig. 33*). In every detail the series of events that leads to the formation of the second spindles is identical with that of the first. Almost every stage in the sequence was carefully examined, and the second was found to be a duplicate of the first series. It will therefore be only necessary to mention the critical stages.

At first the kinoplasmic zone increases uniformly in thickness, and its fibrils run more or less parallel to the nuclear wall.

Very careful search was made for the radial condition described by Osterhout for the second spindle in *Agave*, but nothing like this was found. The fibrils are never radial at this stage.

Having reached a certain thickness the weft no longer increases uniformly, but grows out at irregular intervals from the primary cones of the spindle in identically the same fashion as it does in the formation of the first spindle (*fig. 24*).

SUMMARY.

1. In *Iris* the formation of the spindle is initiated by the transformation of the cytoplasmic reticulum close to the nuclear membrane into a weft of kinoplasmic fibrils, which forms a complete zone about the nucleus.

After increasing to a certain thickness, the zone projects outwards at irregular intervals, forming a series of cones which terminate in sharp points.

The cones apparently develop at the expense of the cytoplasmic reticulum into which they project, and as they grow the fibrils composing them lengthen and converge at the apex.

During the complete formation of the primary cones the nuclear wall persists.

Upon the breaking down of the nuclear membrane the cones fuse until there are two groups of them pointing in opposite directions.

The points at which the cones forming these groups meet at their apices become the poles of the bipolar spindle.

2. In *Disporum* the first indication of the spindle is the formation of a weft of kinoplasmic fibrils which partially surrounds the nucleus. As in *Iris*, the fibrils composing the weft are formed by the transformation of the cytoplasmic reticulum. Unlike *Iris*, the meshes of the weft do not run parallel to the nuclear membrane.

The weft increases irregularly, forming several projections which become the primary cones of the spindle. As they grow outward the cones become sharp-pointed and their fibrils are sharply defined. It is very clear that the kinoplasmic weft is of cytoplasmic origin.

After the cones have developed the nuclear wall breaks down, and the cones unite in two groups to form the bipolar spindle.

3. As in *Iris* and *Disporum*, the spindle in *Hesperaloe* originates from a weft of kinoplasm. The latter completely surrounds the nucleus and is of cytoplasmic origin. As in *Iris*, the fibrils of the weft run parallel to the nuclear membrane.

By growing out at irregular intervals the weft develops a series of sharp-pointed projections which become the primary cones of the spindle.

As the nuclear wall disappears, the cones collect in two groups and fusion at their apices brings about the bipolar condition.

4. In *Hedera*, as division approaches, the cytoplasm close to the nucleus becomes changed into a weft of kinoplasmic fibrils, which forms a zone completely surrounding the nuclear membrane.

This change in the form of the cytoplasm proceeds at intervals in such a way that the kinoplasmic zone appears to grow out in the form of projections. These projections terminate in sharp points and become the primary cones of the spindle.

As the cones grow outward the fibrils composing them become more sharply defined, elongate, and converge at their apices.

The events that follow are essentially the same as those in *Iris*, *Disporum*, and *Hesperaloe*.

The method of spindle formation of the second division is a duplicate of the first.

5. While the various methods of spindle formation described for the higher plants differ in certain respects, the resemblances between others are sufficiently great in warranting a classification of them. The following classification of the types is therefore suggested:

Type 1, represented by *Gladiolus*, *Iris*, *Disporum*, *Hesperaloe*, *Hedera*, *Osmunda*.

Type 2, represented by *Cobaea*, *Passiflora*, *Lavatera*.

Type 3, represented by *Equisetum*.

Type 4, represented by *Agave*.

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EXPLANATION OF PLATES XV AND XVI.

The figures were drawn with the Abbé camera, Zeiss apochromatic immersion obj. 12^{mm}, 1.30 ap., compensating ocular no. 6.

PLATE XV.—FIGS. 1-11. *Iris florentina*.

FIG. 1. A pollen mother-cell; cytoplasm in contact with nuclear membrane transformed into a weft of kinoplasmic fibrils, forming a narrow zone surrounding nucleus.

FIG. 2. Slightly older stage; kinoplasmic weft of considerable but not uniform thickness, preparatory to forming primary cones of spindle.

FIG. 3. Older; outward projections of the weft developed into a distinct sharp-pointed cone. Fibrils composing the cone no longer parallel to the nuclear wall, but directed outward and convergent at apex of cone.

FIG. 4. Like *fig. 3*, but also shows that cones do not develop simultaneously.

FIG. 5. Two cones nearly fully developed; a third beginning.

FIG. 6. Three primary cones of about the same size. Up to this stage nuclear wall is intact, taking no part in formation of kinoplasmic weft; nucleolus also persistent and now vacuolate.

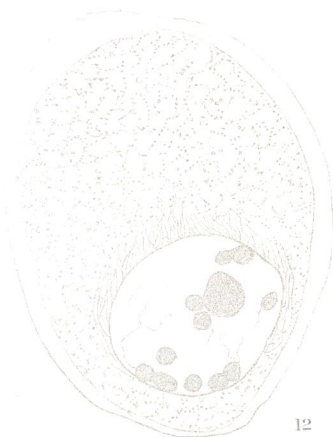
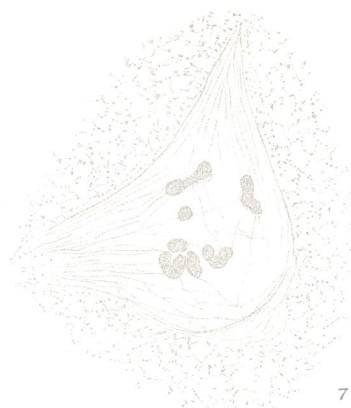
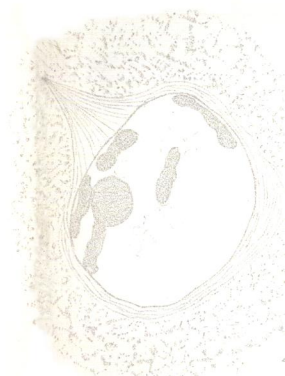
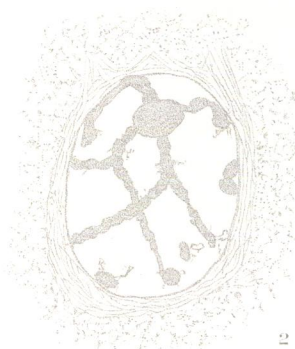
FIG. 7. Nuclear wall and nucleolus have partly disappeared; fibrils at base of cones have grown inward and some have joined chromosomes.

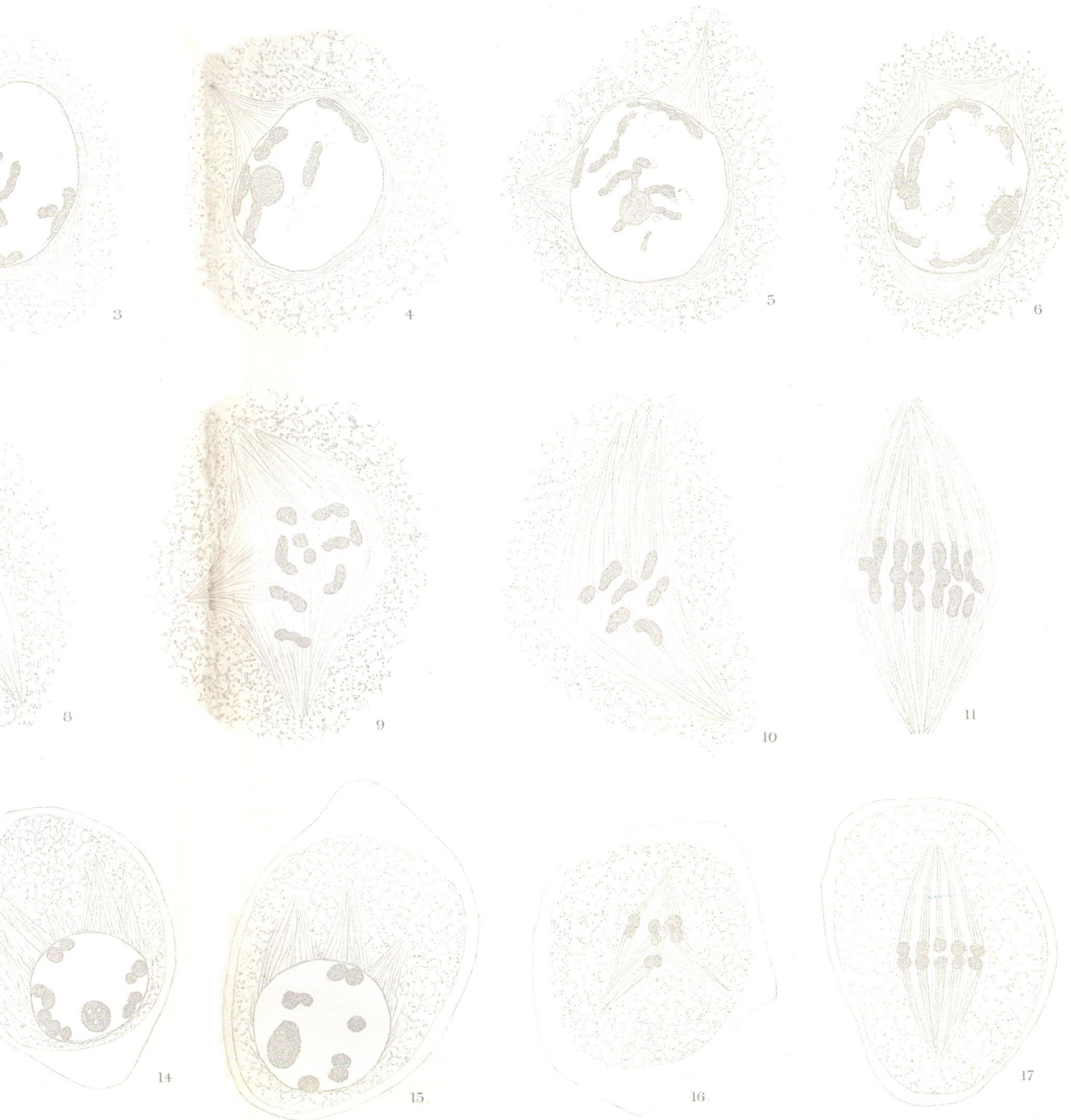
FIG. 8. A characteristic multipolar figure; the three cones have evidently resulted from the union of several primary cones.

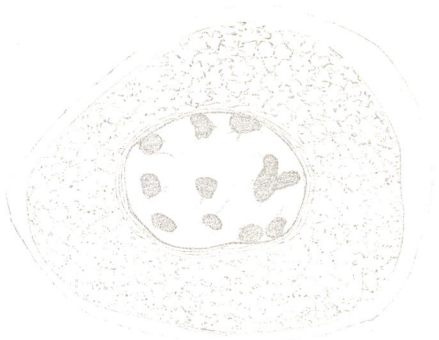
FIG. 9. Somewhat older stage showing further fusion of cones at their apices.

FIG. 10. Cones have united into two groups with their apices pointing in opposite directions, indicating the position of the bipolar spindle.

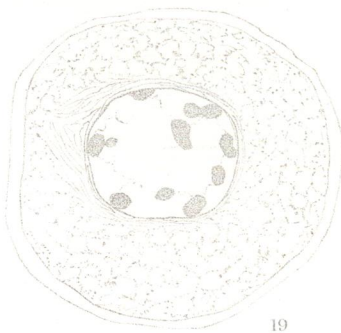
FIG. 11. Mature spindle.







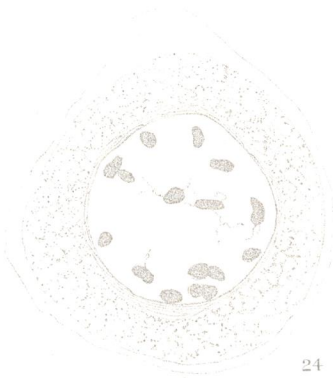
18



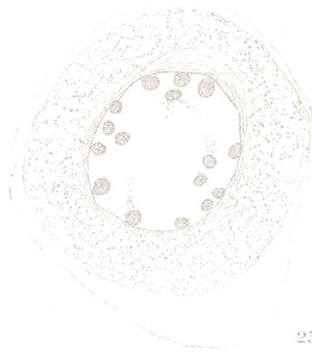
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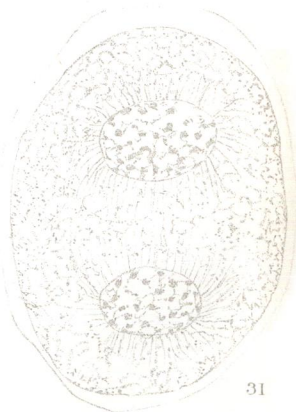
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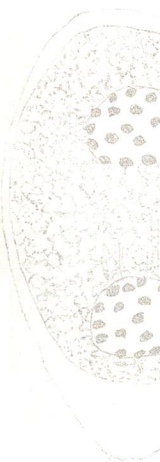
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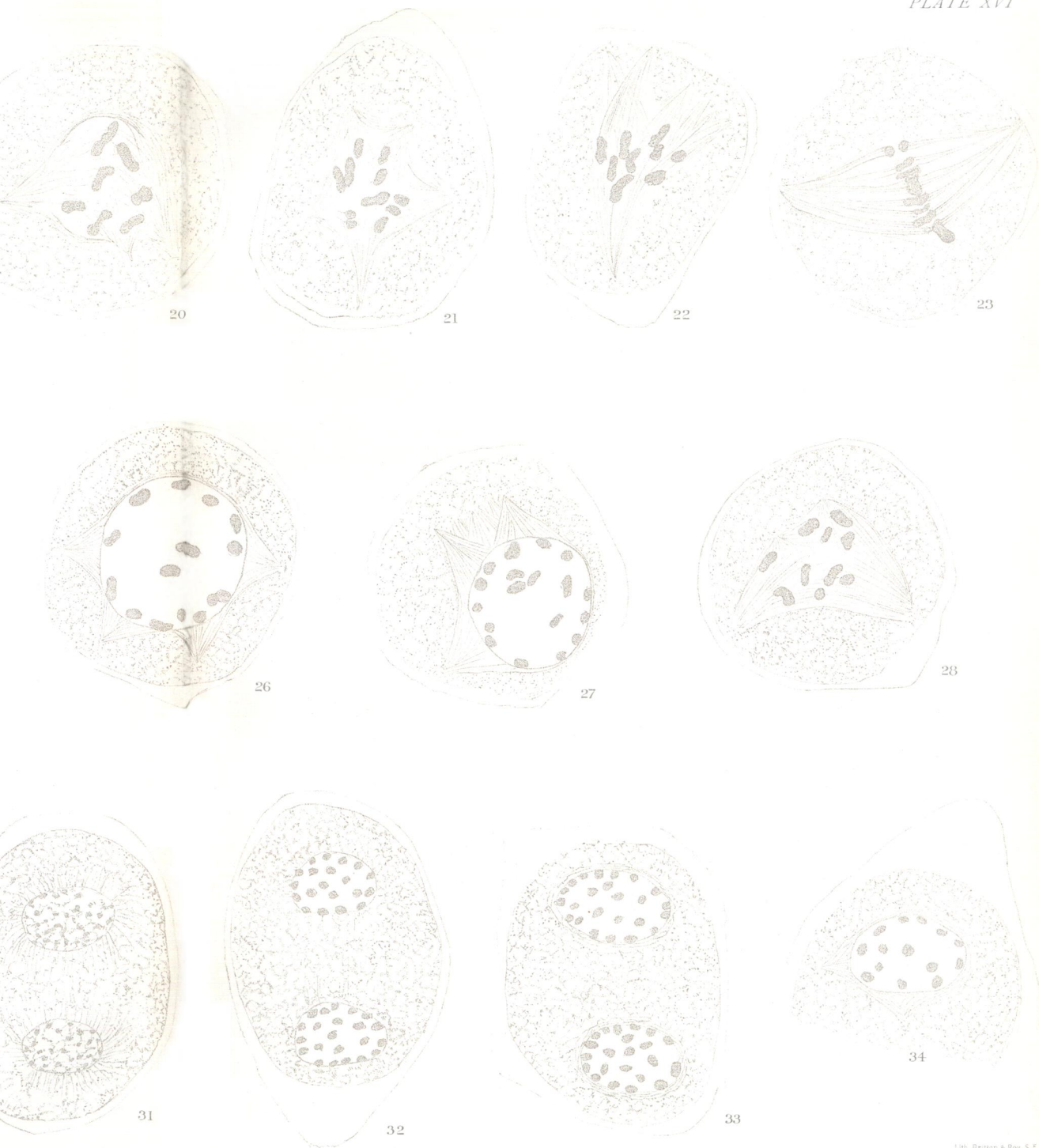


PLATE XV.—FIGS. 12-17. *Disporum Hoekori*.

FIG. 12. Spore mother-cell showing transformation of cytoplasm into a weft of kinoplasmic fibrils not parallel to the nuclear wall.

FIG. 13. Later development of kinoplasmic zone; the weft not uniform but much more abundant on one side of nucleus.

FIG. 14. Weft in the form of irregular sharp-pointed primary cones; their fibrils very much elongated and convergent.

FIG. 15. Fibrils forming cones more independent of one another and more sharply defined.

FIG. 16. Nuclear membrane gone; cones united at apices; fibrils joined with chromosomes; the latter taking position at the equator.

FIG. 17. Mature bipolar spindle.

PLATE XVI.—FIGS. 18-23. *Hesperaloe Davyi*

FIG. 18. Pollen mother-cell, showing formation of kinoplasmic zone quite uniform in thickness.

FIG. 19. Later irregular outward growth of kinoplasmic zone; the meshes composing it point in the direction of the outward projection or primary cone.

FIG. 20. Nuclear wall breaking down; inward growth of fibrils from the base of the cones with which the chromosomes are now in contact.

FIG. 21. Typical multipolar spindle after entire disappearance of nuclear membrane.

FIG. 22. Cones collecting in two groups and uniting at their apices so as to indicate position of bipolar spindle.

FIG. 23. Mature bipolar spindle.

PLATE XVII.—FIGS. 24-34. *Hedera helix*

FIG. 24. Young pollen mother-cell showing early stage in the formation of kinoplasmic weft.

FIG. 25. Later stage of kinoplasmic zone, indicating by one-sided growth the beginning of one of the primary cones.

FIG. 26. Four more fully developed primary cones in the same plane; fibrils composing them much more clearly defined and convergent.

FIG. 27. Somewhat older stage with several cones in the same plane; fibrils very clearly defined and cones almost fully developed.

FIG. 28. Nuclear wall gone; chromosomes in contact with spindle fibrils; cones partially united in two groups indicating future position of bipolar spindle.

FIG. 29. Mature bipolar spindle. As there is a large number of chromosomes, the spindle is very wide at the equator.

FIG. 30. Chromosomes at the poles of spindle; nuclear wall not yet formed around daughter nuclei; many continuous fibrils between the daughter nuclei; also a series of short radiating mantle fibrils, which extend out in all directions from the masses of chromatin.

FIG. 31. Daughter nuclei with membranes; chromatin in spirem condition; continuous fibrils disappearing midway between the daughter nuclei; system of radiating fibrils persistent.

FIG. 32. All fibrils of first spindle have disappeared; chromosomes of daughter nuclei ready for second division.

FIG. 33. New weft of kinoplasmic fibrils forming a zone about each daughter nucleus, the first indication of spindles for second division.

FIG. 34. Weft growing out from one of the daughter nuclei to form primary cones of second spindle.